Chapter 7: Causal Criteria for Assessing Endocrine Disruptors—A Proposed Framework

7.1 Introduction

To create an objective and unbiased assessment of the hypothesis that chemicals with endocrine activity may be having adverse effects on laboratory animals, wildlife populations, and humans, all of the relevant information needs to be considered in an organized and structured manner. The challenge of this task stems from the vast number of studies conducted, the improbability that a single study could provide all the necessary information to link an exposure scenario to a particular health outcome in wildlife or humans, and the diverse circumstances (e.g., varied experimental conditions, numerous end points) from which data have been generated. Therefore, this chapter proposes the use of an organized framework, based on criteria modified by Bradford-Hill (1965), Fox (1991), and Ankley et al. (1997), to be used in the assessment of relationships between exposures to potential EDCs and altered health outcomes. It is important to recognize that the goal of this approach is not to provide point estimates of the association, as is case for quantitative meta-analyses (Greenland, 1998), but to reconcile different results from different studies as in done in qualitative meta-analyses (Cook et al., 1994). Examples of studies are included to illustrate how this framework can be used to assess causal relationships between "exposure and effect" and whether these associations involved endocrine-mediated events (see Tables 7.1 and 7.2). These examples are described in greater detail in other chapters of this assessment.

This structured, framework approach acknowledges that 1) there are a number of scientific uncertainties, 2) a degree of scientific judgment is involved, and 3) assessments are likely to change as additional information becomes available. This approach can identify key data gaps and research needs (see Chapter 8) that may reduce the uncertainties associated with the study of EDCs. Also, it should be noted that these assessments are qualitative determinations of the current overall state of the science. They are not quantitative risk assessments that relate specific exposure situations to probabilities of adverse effects. The objective of the framework is to provide a tool to evaluate the myriad of divergent and, at times, discordant data sets. In doing so, key research gaps may be highlighted and more informed assessments might be promoted in the future. By necessity, this approach is most useful when applied to the examination of a large body of evidence in the search for cause-and-effect relationships. It is less useful for identifying new signals in the environment that could be suggestive of endocrine disruption, although the establishment of the framework should still serve to guide research that could build the case for causality by pinpointing the key gaps in our knowledge.

List of Abbreviations

AhR Arylhydrocarbon receptor
DDE Dichlorodiphenyl dichloroethylene
DDT Dichlorodiphenyl trichloroethane
EDCs Endocrine-disrupting chemicals
GLEMEDS Great Lakes embryo mortality, edema, and deformity syndrome
Ig Immunoglobulin
PCBs Polychlorinated biphenyls
PCDDs Polychlorinated dibenzodioxins
PCDFs Polychlorinated dibenzofurans
T_4 Thyroxine
TBT Tributyl tin
TCDD 2,3,7,8-Tetrachlorodibenzyl-*p*-dioxin
TSH Thyroid-stimulating hormone
USA United States of America

7.2 Elements of the Proposed Framework

The framework begins with a clear statement of the *hypothesis* under examination, which contains two distinct elements. First, the outcome of concern (e.g., a specific human disease or status of an ecological species) is linked to a putative stressor that is acting on the individual or population. Second, exposure to the stressor results in endocrine-mediated events that ultimately result in the outcome of concern. These elements need to be clearly stated in order to evaluate the scientific evidence regarding their potential relationship. The evaluation of the scientific evidence utilizes five aspects: 1) temporality, 2) strength of the association, 3) consistency of the observations, 4) biological plausibility of the effect, and 5) evidence for recovery following diminution of the stressor. The aspect of specificity of the association, a traditional component of causality in the epidemiological setting, is not included in this framework because some of the examined outcomes (e.g., semen quality) are quite apical in nature and influenced by many factors, and the component of biological plausibility covers the linkage between the mechanism of action and the outcome (e.g., estrogen mimics and vitellogenin induction in fish) and hence deals implicitly with specificity.

1) The aspect of *temporality* explores whether the presumed cause of the outcome of concern preceded the appearance of altered physiological states, rates of disease, or population health. Although information regarding the onset of exposure is often lacking, a few examples are included in which the temporal pattern of exposure precedes the observed effect.

2) The aspect of *strength of the association* examines a) the incidence rate of the outcome in a population, b) the extent to which other known risk factors may have contributed to this incidence, c) the risk that could be attributed to the exposure of concern, and d) the shape of the dose–response curve as determined either from laboratory or population-based studies.

3) The aspect of *consistency of the observations* examines how frequently similar or dissimilar conclusions are reached in the literature and discusses any apparent discrepancies. It also evaluates whether results came from multiple geographical areas, whether multiple species would be expected to react in a similar fashion, and whether studies employed similar dosages.

4) The aspect of *biological plausibility* examines multiple areas of research (e.g., basic aspects of biology, embryology, endocrinology, population dynamics, chemical/physical properties, etc.) that help determine the mechanism of action for the compounds of concern. Consideration of a substance's mechanism is critical because this criterion is central to the overall assessment of whether or not a substance is deemed to be an "endocrine disruptor." In this assessment, a substance meets the operational definition of an endocrine disruptor if it "alters the function of the endocrine system and consequently causes an adverse health effect in an intact organism, or its progeny, or (sub)populations."

5) The aspect of *evidence of recovery* examines whether the occurrence of the adverse outcome is reversible upon diminishment or cessation of the suspected exposure. When examining the issue of recovery, it is important to note that some effects may be developmentally imprinted, and hence recovery may only occur in

Table 7.1 - Illustrative Examples

Statement of Hypothesis		Evaluation Factor					Overall Strength of Evidence	
Outcome	Stressor	Strength of Temporality	Association	Biological Consistency	Plausibility	Recovery	For Hypothesis	For EDC Mechanism
Endometriosis in humans	TCDD, PCBs	ND	*	*	*	ND	Weak	Moderate
Impaired neurobehavioral development in humans	PCBs	****	***	***	***	ND	Moderate	Moderate
Perturbed immune function in humans	PCBs, TCDD	***	****	**	**	*	Moderate	Weak
Incidence of breast cancer in humans	DDT, DDE, and PCBs	*	*	*	**	ND	Weak	Weak
Imposex in marine gastropods	TBT	****	****	****	***	****	Strong	Strong
Decreased reproductive function in Baltic seals	PCBs	***	**	***	***	****	Strong	Moderate
GLEMEDS in birds	Polychlorinated halogens (PCBs)	****	****	****	****	****	Strong	Weak
Egg shell thinning in colonial waterbirds	DDE and other DDT metabolites	****	****	****	***	****	Strong	Moderate
Reproductive abnormalities in Lake Apopka alligators	Dicofol and agricultural pesticides	****	***	***	***	**	Moderate	Moderate
Developmental abnormalities and reproductive failure in Lake Ontario lake trout	Dioxins and coplanar PCBs	****	****	***	****	****	Strong	Weak
Vitellogenin induction in fish exposed to sewage treatment plant effluents in England	Estrogenic contaminants	****	****	***	****	**	Strong	Strong
Reproductive alterations in fish exposed to bleached Kraft mill effluent in Ontario	Bleached Kraft mill effluent	****	****	***	****	***	Strong	Strong

ND, no relevant data. This table summarizes the overall strength of evidence for each criterion of the framework (evaluation factors) developed to assess the potential effects of EDCs. Each criterion has been ranked from weak (*) to strong (****), and each element of the hypothesis (outcome, stressor, and EDC mechanism) is evaluated as either weak, moderate, or strong.

Table 7.2 Illustrative Examples (Status and Trend Data Only)

Statement of Hypothesis		Evaluation Factor					Overall Strength of Evidence		
Outcome	Stressor	Temporality	Strength of Association	Consistency	Biological Plausibility	Recovery	For Outcome	For Hypothesis	For EDC Mechanism
Reduction in semen quality and testis function in humans	Estrogenic and antiandrogenic chemicals	ND	ND for association * for effect	ND for exposure * for effect	***	ND	Weak	ND	Weak
Limb malformations in North American frogs	Unknown chemical etiology	ND	ND for association ** for effect	ND for exposure * for effect	**	ND	Strong	Weak	Weak

ND, no relevant data. This table summarizes the overall strength of evidence for each criterion of the framework (evaluation factors) developed to assess the potential effects of EDCs. Each criterion has been ranked from weak (*) to strong (****), and each element of the hypothesis (outcome, stressor, and EDC mechanism) is evaluated as either weak, moderate, or strong.

subsequent generations, or may even express themselves in subsequent generations that have not in themselves been exposed to the stressor.

7.3 Overall Strength of Evidence

The final part of the framework, *overall strength of evidence*, makes an evaluation regarding the relationship between an outcome of concern and exposure to a substance and whether or not these associations involve endocrine-mediated mechanisms. These concluding remarks are drawn from the five criteria described above.

7.4 Illustrative Examples—Status and Trends Observations

7.4.1 Semen Quality and Testis Function in Humans

Hypothesis: Global reductions in human semen quality over time are related to increasing exposure to estrogenic, antiandrogenic (identity unknown), or other as yet unidentified chemicals, during critical phases of testicular development.

Temporality: A number of studies from different parts of the world have shown significant declines in sperm count and semen volume in men over time. However, none of the studies of semen quality have included prenatal, childhood, or adult exposure assessments for estrogenic or antiandrogenic chemicals, and the decline began before the use of industrial chemicals became widespread, especially if one considers the effect to be due to exposures during fetal or early postnatal life.

Strength of association: There are no human data that directly address the proposed cause-and-effect relationship. Concerning strength of effect, the meta-analysis showed a decline in semen quality of around 50% over 50 years, or 1.5% per year for the USA and 3.5% per year for Europe.

Consistency: There are no data relating to consistency of effect with exposure to chemicals of concern. A meta-analysis of studies published between 1938 and 1990 from 20 countries showed declines in sperm count and semen volume in men over time. Of the subsequent longitudinal studies in single centers, 10 are consistent with a decline, 6 are consistent with improvement, and 8 show

no change in semen quality over time. The many potential confounders in semen quality studies (e.g., differing population characteristics, methods of semen collection and analysis) could explain the inconsistencies. Two "time to pregnancy" (fecundity) studies have also produced results that are not consistent with decreased semen quality (i.e., they have not observed a decline in fertility among couples), although it should be noted that many factors influence fertility.

Biological plausibility: Endogenous estrogens control testis development. However, prenatal exposure to pharmacological estrogens, including diethylstilbestrol, in humans is not associated with effects on fertility. Support for biological plausibility comes from human data for incidence trends in developmentally related end points (i.e., testicular cancer and male reproductive tract abnormalities). Further supportive evidence comes from experimental animal data showing adverse effects on male reproductive tract development and adult testicular function with exposure to estrogenic and antiandrogenic chemicals (e.g., estradiol, nonylphenol, methoxychlor, vinclozolin, phthalates, TCDD). The prenatal and perinatal periods are particularly vulnerable to disturbances of male reproductive tract development by these chemicals, whereas higher doses are required to affect testis function in the case of adult exposure.

Recovery: No relevant data.

Overall strength of evidence

For outcome, the evidence is judged to be weak. A global trend for declining semen quality is not supported by current data. Some studies show declines in certain regions or cities, whereas others have not found a decline, suggesting there may be regional trends but not a global trend. There is no evidence relating to the strength of the hypothesis because of the lack of exposure data.

There are no human data to support an EDC-related mechanism. However, the biological plausibility of the hypothesis remains strong, based on information from clinical experience and experimental systems.

7.4.2 Limb Malformations in North American Frogs

Hypothesis: Exposure to chemicals that influence endocrine function contributes to the increased prevalence of limb malformations in North American frog populations

Temporality: There has been a recent increase in the number of observations of deformed frogs captured over wide geographic regions of North America, suggesting that the incidence of such effects is increasing. The temporality is difficult to reconcile in the context of a general global reduction in chemical exposure.

Strength of association: The strength of association with chemical exposure is weak.

Consistency: In the absence of information on the stressors responsible for malformations, it is difficult to assess whether response are consistent across time or at different sites.

Biological plausibility: Current knowledge of the involvement of endocrine processes in the development of amphibians provides the underlying mechanistic basis that the effects seen in frogs may be a consequence of exposure to chemicals found in the environment. However, cause-and-effect relationships must be further examined.

Recovery: There are no data available.

Overall strength of evidence

For the outcome of concern, there is considerable evidence that the incidences of malformations in frog populations are high and/or rising. At this time, the evidence that there is a chemical etiology mediating these malformations or that this involves effects on endocrine function is weak.

7.5 Illustrative Examples—Nonstatus and Trend-Type Observations

7.5.1 Endometriosis in Humans

Hypothesis: Endometriosis in women is related to endocrine disruption mediated by exposure to TCDD and/or PCBs.

Temporality: Not assessable. Endometriosis is a common disease in women, but there are few data on temporal trends. Exposure to dioxins and PCBs is ubiquitous.

Strength of association: There are no estimates of the proportion of endometriosis attributable to TCDD/PCBs. The only case–control study gave an odds ratio of 7.6 (95% confidence interval, 0.87–169.7).

Consistency: Two studies reported an association between serum levels of TCDD and endometriosis, one with no dose response. One study reported an association between PCB exposure and endometriosis. Another study reported no association between endometriosis and serum PCBs or dioxins. No association was found in Seveso women exposed to high levels of TCDD.

Biological plausibility: There is a clear dose–response relationship between endometriosis and endogenous estrogens and exposure to pharmacological estrogens, but TCDD can oppose the effects of estrogen. Conflicting evidence comes from studies of endometriosis in monkeys; one positive for TCDD exposure, one showing a bimodal dose response for TCDD, and one negative for PCB exposure. In mice (but not rats), surgical induction of endometriosis is enhanced by exposure to relatively high doses of TCDD or 4-chlorodiphenyl.

Recovery: No relevant data.

Overall strength of evidence

Relative to the hypothesis of an association between a stressor and an outcome, evidence is judged to be weak because of conflicting data from humans and animals, lack of association in women exposed to high amounts of TCDD, and antiestrogenic effects of TCDD.

In humans, occurrence of endometriosis shows dependency on estrogen–progesterone balance, suggesting that an EDC-related mechanism may be possible.

7.5.2 Impaired Neurobehavioral Development in Humans

Hypothesis: Impaired neurobehavioral development in children is related to endocrine disruption mediated by exposure to PCBs.

Temporality: Impaired neurobehavioral development has been observed in association with prenatal and possibly early postnatal exposure to PCBs.

Strength of association: A range of adverse, persistent effects have been observed in offspring of mothers exposed to relatively high levels of PCBs (Yusho and Yu-Chen poisoning incidents). More subtle, less persistent effects have been observed in certain populations exposed to lower PCB levels.

Consistency: Although there are several studies reporting neurological effects in children exposed to low levels of PCBs, the outcomes measured in studies have showed some variability. In reviewing the evidence, consideration was given to the complexity in measuring neurological function in children, the different populations in which effects were reported, and the fact that the studies were not designed to be replicates of one another. In addition, the timing of exposure may be critical but has not yet been characterized, particularly with respect to postnatal exposure.

Biological plausibility: PCBs and other agonists of the AhR are known to interfere with thyroid hormones and sex hormone action,

which are known to be critical for normal brain development. PCBs are known to have hypothyroid actions at exposure levels in certain populations. Evidence from experimental animal studies of neurobehavioral effects and elevated brain thyroxine deiodinase levels in offspring exposed to PCBs during gestation also supports biological plausibility.

Recovery: No relevant data.

Overall strength of evidence

Relative to the hypothesis of an association between a stressor and an outcome the evidence is judged to be moderate. Several human studies show a range (severe to minor) of adverse effects but within the same continuum of motor and mental developmental delays or impairments. There is appropriate temporality in relation to PCB exposure, a broad dose response across studies (but not necessarily within studies), and reasonable consistency, except with respect to the possible contribution of postnatal exposure. Interpretation is complicated by incomplete exposure measures in some studies (maternal serum and/or cord serum and/or milk).

There is limited direct human evidence of an EDC-related mechanism, with maternal serum/milk PCB/PCDD/PCDF/TCDD levels reported to be negatively associated with infant T_4 levels and positively associated with infant TSH levels, but T_4 and TSH still within their normal clinical range. Overall, the evidence is judged moderate.

7.5.3 Perturbed Immune Function in Humans

Hypothesis: Perturbations in immune function via changes in endocrine function are mediated by exposures to PCBs and TCDD.

Temporality: Most human data are derived from studies of *in utero* and/or accidental exposure. The outcomes were assessed after the exposure had occurred. However, no data are available regarding the presence of symptoms and/or baseline measurement of immune function cells prior to exposure.

Strength of Association: In children, *in utero* exposure to PCBs and TCDD is associated with abnormal measures of immune function cells and serum antibodies. In children exposed during the pre- and postnatal period, a higher prevalence of respiratory symptoms and other infectious diseases (but a lower prevalence of allergic disease) was observed. These observations span a range of exposure from very high (Yu-Cheng cohort) to background levels (Dutch breastfeeding study). In adults, alterations of immune function are observed in all but one study following both accidental and occupational exposure; no data are available for general environmental levels of exposure.

Consistency: At high levels of exposure, there is ample evidence for perturbed immune function in both children and adults. At lower levels, there is only one study in children and no data in adults.

Biological plausibility: *In vitro* and *in vivo* data suggest that TCDD induces AhR-mediated thymic atrophy. TCDD may also deplete thymocytes through either apoptosis or direct action on the bone marrow. These data qualitatively support the human studies.

Recovery: Longitudinal data from the Yusho cohort indicate decreased levels of serum IgA and IgM 2 years after exposure ceased, although an increased prevalence of respiratory symptoms persisted for longer times.

Overall Strength of Evidence

Relative to the hypothesis of an association between a stressor and an outcome, the evidence is deemed moderate. Most studies in children and adults indicate moderate associations between high levels of exposure to TCDD and measures of immune function. Evidence for perturbed immune function following exposure to lower levels of PCBs is limited to one study, and more evidence is clearly needed.

Regarding an EDC-related mode of action, the overall assessment is weak. Both *in vivo* and *in vitro* data suggest that TCDD perturbs the activity of the thymic epithelium possibly due to AhR-mediated thymic atrophy.

7.5.4 Incidence of Breast Cancer in Humans

Hypothesis: Increased incidences of breast cancer are caused by exposure to organochlorine chemicals (e.g., PCBs, DDT, and metabolites) possessing estrogenic activity.

Temporality: Little information is available on the patterns of organochlorine exposure from birth to menopause in case–control studies. Because organochlorines are biologically persistent, the current exposure measures may represent past exposure. The timing and magnitude of the exposure during the full life span may be critical in order to detect effects on breast cancer risk.

Strength of Association: Most studies show no association between breast cancer and organochlorine exposures; the few positive studies are weak.

Consistency: For DDT and its metabolites, there are seven reported positive associations that are statistically significant out of 34 studies in total. For PCBs there are five reported positive associations that are statistically significant out of 24 studies in total.

Biological plausibility: There is strong evidence that physiological levels of naturally occurring estrogens can contribute to breast cancer risks in women and that the cumulative lifetime exposure to endogenous estrogens correlates with the incidence of breast cancer in populations. Additional exposure to estrogenic chemicals could therefore plausibly increase the risk of disease. However, the potency of "organochlorine" estrogens is low compared with endogenous hormones and phytoestrogens, and therefore, any added risks from PCB or DDT exposure would be very small relative to the contributions of endogenous estrogens and probably not detectable in case–control studies.

Recovery: No relevant data

Overall Strength of Evidence

For breast cancer as an outcome, the evidence that the incidences are increasing is moderate based on several valid and well-conducted surveys. Breast cancer screening practices and early detection may have contributed to the reported increases.

In terms of stressors, the evidence is weak in support of the hypothesis that exposure to PCBs, DDT, and other organochlorines contribute to increased risk based on the lack of consistency of the results, weak associations, and questions of biological plausibility. The evidence is also weak for an EDC mode of action.

7.5.5 Imposex in Marine Gastropods

Hypothesis: TBT originating from antifouling paints used to treat boat hulls induces a form of pseudohermaphroditism (termed imposex) in female gastropods by an endocrine-disrupting mechanism.

Temporality: The use of TBT has been associated with increased incidence of imposex and population declines.

Strength of association: The frequency of imposex and the degree of penis development in females are related to the degree of TBT exposure. Laboratory studies have confirmed the effects of TBT on imposex in neogastropod mollusks. A related condition termed intersex, which involves TBT-induced changes in the oviduct, occurs in littrinid gastropods.

Consistency: There is strong evidence of worldwide effects on gastropod populations. Imposex is a generalized response in marine gastropods exposed to TBT, because effects have been seen in over 100 species in almost 50 genera.

Biological plausibility: The effects of TBT have been reproduced in controlled laboratory studies. Current understanding of the mechanism of action of TBT is incomplete, although it appears to have an endocrine basis related to mechanisms that contribute to the elevation of androgen levels (inhibition of aromatase).

Recovery: Banning the use of TBT in antifouling paints has been effective in reducing environmental concentrations of TBT and a corresponding decrease in the incidence of imposex and/or increased reproductive success of previously affected gastropod populations.

Overall strength of evidence

Relative to the hypothesis of an association between a stressor and an outcome, the evidence that TBT affects sexual development and reproduction in female gastropods represents one of the strongest case studies showing that exposure to environmental chemicals contributes to population-level impacts.

For an EDC-related mode of action, the evidence is considered strong in that alterations in aromatase activity have been implicated in the alteration of steroid hormone profiles (elevated androgen levels).

7.5.6 Decreased Reproductive Function in Baltic Seals

Hypothesis: Exposure to persistent organochlorine contaminants, including PCBs derivatives, contributes to reproductive toxicity in Baltic seals through an endocrine dependent mechanism.

Temporality: High levels of persistent organochlorine contaminants are strongly correlated with reduced reproductive success of Baltic seal populations. Exposures to organochlorine contaminants are consistent with the induction of a range of abnormalities including uterine alterations that are thought to contribute to sterility and lowered reproductive success.

Strength of association: In general, the link between adverse reproductive outcomes and chemical etiology is weak. This is further complicated by the presence of a range of pathological lesions and immune function alterations where the linkages with reproductive outcome is unknown.

Consistency: It has been difficult to evaluate the consistency of responses in the field setting when so many factors are variable. Nevertheless, observations in marine mammals on a global scale provide evidence that Baltic seals may be susceptible to alterations in reproduction.

Biological plausibility: The results of semi-field studies with Baltic seals and harbor seals provide general links with organochlorine exposure and reproductive outcome, but there are complications in establishing cause-and-effect relationships because of inadequate study designs.

Recovery: There is little opportunity to examine the consistency of the response and recovery over time. In general, reproduction has been improving as the levels of contaminants decline.

Overall strength of evidence

Relative to the outcome of concern, there is considerable evidence that the reproductive success of Baltic seal populations has been impacted and that these exposures have altered adrenal gland function in members of the exposed population. However, because the link between altered adrenal function and reproductive impairment has not been clearly established, the overall evidence pertaining to the existence of an EDC-related mode of action is moderate.

7.5.7 GLEMEDS

Hypothesis: Developmental abnormalities and embryo mortality in colonial fish-eating water birds in the Great Lakes of the USA are a consequence of exposure to persistent organochlorine compounds including PCBs, which act though an endocrine-dependent mechanism.

Temporality: Several fish-eating bird species (e.g., double-crested cormorants, herring gulls, Forster's and common terns, bald eagles) in the Great Lakes experienced severe population declines from the 1940s to early 1970s. This was associated with exposure to high levels of persistent organochlorine compounds. Incidences of GLEMEDS have decreased as levels of α compounds in the environment have decreased.

Strength of association: Ecoepidemiological studies point to the syndrome being mediated by exposure to persistent organochlorine compounds, particularly PCBs, which bioaccumulate in the egg. Laboratory experiments have been instrumental in establishing the relationship between exposure to AhR agonists and the GLEMEDS syndrome.

Consistency: The similar pattern of response seen in several avian species at different sites within the Great Lakes adds to the weight of evidence linking exposure and outcome. Similar responses were observed in chickens exposed to PCDFs and PCDDs.

Biological plausibility: Several studies confirm that early life stages are particularly sensitive to chemicals that act through the AhR.

Recovery: There have been reductions in the extent and severity of symptoms of GLEMEDS with reductions in environmental exposures of organochlorine compounds.

Overall strength of evidence

The evidence for a causal relationship between the postulated stressor and the GLEMEDS outcome is strong (as demonstrated in several areas, with supported evidence from laboratory experiments).

Because the outcome (embryonic edema and mortality) is not necessarily related to alterations in endocrine function, and because of the lack of mechanistic studies in model systems, there is considerably less certainty that an EDC mode of action is involved in this observation.

7.5.8 Eggshell Thinning in Colonial Waterbirds

Hypothesis: Eggshell thinning caused by exposure to DDE results in cracked or broken eggs and other adverse reproductive effects through an endocrine-mediated mechanism.

Temporality: During periods of high use of DDT as an insecticide in North America and Europe, DDE-induced eggshell thinning nearly resulted in the extinction of several avian species.

Strength of association: The strength of association between DDE exposure and effects on eggshell thickness is strong, with similar effects being seen in both North American and European studies.

Consistency: Adverse responses to DDE have been observed in a range of species at multiple sites. The sensitivity to DDE-induced eggshell thinning varies markedly among avian species, and different mechanisms may contribute to the types of eggshell defects seen in different species.

Biological plausibility: Numerous laboratory-based and *in vitro* studies confirm the relationship between DDE exposure and adverse reproductive outcome; however, the laboratory studies have yielded somewhat inconsistent results regarding the mode of action.

Recovery: Many species that are sensitive to eggshell thinning have experienced dramatic population increases as a result of reduced exposure to DDT and its metabolites.

Overall strength of evidence

Relative to the outcome of concern, there is strong evidence that eggshell thinning results from exposure to DDE.

There continues to be uncertainty with respect to the precise mechanism of action of DDE and the extent to which this involves alterations in endocrine function. The likelihood that the outcome involved an EDC mode of action is considered moderate, due primarily to the relationship with altered prostaglandin synthesis in the mucosa gland of sensitive species.

7.5.9 Reproductive Abnormalities in Lake Apopka Alligators

Hypothesis: Reproductive tract and endocrine abnormalities observed in Lake Apopka alligators are a result of exposure to chemicals originating from a spill of the pesticide dicofol (including its metabolic or environmental breakdown products) or ongoing agricultural practices.

Temporality: Reproductive failure leading to reductions in the numbers of neonate and juvenile alligators, developmental abnormalities of the reproductive tract and the male phallus, and abnormal sex steroid levels were observed in Lake Apopka alligators in the years following a chemical spill. These response patterns have persisted for over 15 years following the chemical spill.

Strength of association: This is rated as strong because similar response patterns have persisted over time.

Consistency: There is a high degree of consistency in responses seen within Lake Apopka (in general, the response patterns observed in other locations are highly variable).

Biological plausibility: It is evident that alligators in Lake Apopka have been exposed to chemicals that are known to interact with endocrine receptors or contribute to reproductive toxicity. However, data on cause-and-effect relationships and laboratory-based exposure–response studies are limited.

Recovery: Although there has been some indication of a reduction in the severity of developmental abnormalities and a gradient of responses has been observed within Lake Apopka, there are limited data available with respect to recovery.

Overall strength of evidence

Regarding the outcome, there is a relatively strong indication of adverse effects of chemical exposure on the alligator population in Lake Apopka.

Although there are supporting laboratory data regarding particular chemical stressors and altered endocrine status in the alligator embryo, the current understanding of whether an EDC-related mode of action is responsible is weak.

7.5.10 Vitellogenin Induction in Fish Exposed to Sewage Treatment Plant Effluents in England

Hypothesis: Estrogenic compounds in the effluents from sewage treatment plants throughout England contribute to increases in vitellogenin production and intersex in fish living in the receiving environment.

Temporality: Both vitellogenin production in males and the incidence of intersex in fish are highest immediately downstream of sewage treatment discharges and generally decrease with distance downstream. Caging of naive fish in the vicinity of sewage effluent discharge provides evidence for the strength of association and outcome, at least in terms of vitellogenin production.

Strength of association: Both *in situ* exposure of caged fish and exposure of fish to sewage effluents in the laboratory provide confirmation that effects on vitellogenin production and gonad development are mediated by chemicals originating in the sewage effluent. Other studies at various locations throughout Europe and North America have confirmed the link between sewage treatment plan effluents and vitellogenin induction; far less is known regarding effects on gonadal development.

Consistency: This is rated high based on the temporal patterns of response and the consistency between different geographic areas.

Biological plausibility: Laboratory experiments have established that estrogenic compounds are responsible for increased vitellogenin production and contribute to effects on gonadal development that may lead to intersex. Chemical fractionation studies have confirmed that sewage effluents are significant sources of estrogens (synthetic estrogens and industrial chemicals with estrogenic activity) and that these are present in amounts that are likely to mediate the observed biological effects. There is variability in the concentration response to vitellogenin induction by estrogens in male fish, and species may respond differently to similar exposure levels.

Recovery: Little is known of the long-term effects following exposure to estrogenic compounds.

Overall strength of evidence

For the outcome of vitellogenin induction in male fish downstream of sewage treatment plants, several well-conducted studies convincingly demonstrate a causal relationship.

Relative to an EDC mode of action, environmental monitoring information, laboratory studies, and research on biological plausibility indicate a strong likelihood that an EDC mode of action is involved. The presence of estrogenic compounds in sewage treatment effluents represents one of the best examples illustrating the linkage between exposure to an EDC and an outcome.

7.5.11 Developmental Abnormalities and Reproductive Failure in Lake Ontario Lake Trout

Hypothesis: Exposure to TCDD and coplanar PCBs contributes to mortality during early development and reduced reproductive success in Lake Ontario lake trout through an endocrine-mediated mechanism.

Temporality: Lake trout populations in Lake Ontario declined precipitously during the period when environmental levels of persistent bioaccumulative organochlorine chemicals were highest.

Strength of association: Laboratory studies have shown that exposure to AhR agonists induce blue-sac disease, which was responsible for early-life-stage mortality in the embryos of artificially spawned lake trout. Subsequent retrospective studies (based on measured PCB, PCDF, and PCDD residues in dated sediment cores) have established a strong relationship with the observed historical trends in lake trout reproduction, including recent signs of successful reproduction. Collectively, these results confirm that AhR agonists are primary contributors to early-life-stage mortality and adverse population level impacts.

Consistency: In addition to limited field observations, laboratory studies have established a linkage between dioxin equivalent concentrations and outcome.

Biological plausibility: Laboratory-based studies confirmed that the early life stage is sensitive to AhR agonists, including TCDD and coplanar PCBs, and that the observed pathology (e.g., blue-sac disease) was consistent with observations in embryos from field-collected lake trout.

Recovery: Recent studies have shown that there are reductions in the incidence of blue-sac disease in lake trout, associated with a decline in dioxin equivalent concentrations in Lake Ontario.

Overall strength of evidence

Both field studies and laboratory research provide ample evidence that the outcome of early life stage mortality in lake trout is related to exposure to AhR agonists, including TCDD and PCBs.

Despite the strong association between the stressor and the outcome, experimental studies have yet to establish an EDC-related mode of action.

7.5.12 Reproductive Alterations in Fish Exposed to Bleached Kraft Pulp Mill Effluent in Ontario

Hypothesis: Chemicals present in the effluent from a bleached Kraft pulp mill at Terrace Bay, Ontario, contribute to endocrine dysfunction and delayed reproduction in white sucker fish in the surrounding environment.

Temporality: A series of studies have shown that white sucker fish exposed to the effluent from a bleached Kraft pulp mill at Terrace Bay, Ontario, exhibited changes in reproductive development, including delayed sexual maturity, reduced gonadal growth, and alterations in plasma sex steroid hormone levels. Studies with caged fish in effluent or with fish exposed to effluent in the lab provide evidence of a temporal link between exposure and changes in sex steroid levels.

Strength of association: Studies examining fish downstream of other pulp mills in Canada and Sweden support the hypothesis that chemicals in the effluent contribute to adverse reproductive responses. Fish exposed to effluent from pulp mills also exhibited adverse reproductive effects.

Consistency: This is rated high, because similar responses have been observed over a 10-year period (irrespective of changes in effluent treatment and bleaching technologies).

Biological plausibility: Bleached Kraft mill effluent is a complex mixture of chemicals that make it difficult to identify specific bioactive compounds. Evidence that fish living in these surroundings are exposed to endocrine-active chemicals comes from studies showing that they rapidly accumulate ligands for sex steroid receptors (androgen, estrogen, and sex steroid binding protein) and the AhR. Both lab and field studies provide evidence of adverse effects being linked to effluent exposure.

Recovery: Reproductive endocrine changes are diminished in fish collected during periods of reduced effluent discharge (e.g., mill shutdown). There is also a rapid decrease in concentrations of endocrine-active ligands in the fish following transfer to clean water.

Overall strength of evidence

For the outcome of altered reproductive outcome in fish living in the vicinity of a bleached Kraft mill, there is compelling evidence that chemicals in the effluent are responsible for changes in endocrine function and reproductive performance of fish.

Although the active compounds responsible for the biological effects have not been identified, the findings are consistent with an EDC mode of action. The evidence of altered steroid receptor function in exposed fish is deemed strong.